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Utility of Commonly Used Commercial Human Chorionic Gonadotropin Immunoassays in the Diagnosis and Management of Trophoblastic Diseases

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Background: **Patients with trophoblastic diseases produce ordinary and irregular forms of human chorionic gonadotropin (hCG; e.g., nicked hCG, hCG missing the β-subunit C-terminal segment, hyperglycosylated hCG, and free β subunit) that are recognized to differing extents by automated immunometric hCG (or hCGβ) assays. This has led to low or false-negative results and misdiagnosis of persistent disease. False-positive hCG immunoreactivity has also been detected, leading to needless therapy for trophoblastic diseases. Here we compare seven commonly used hCG assays.**

Methods: **Standards for five irregular forms hCG produced in trophoblastic diseases, serum samples from 59 patients with confirmed trophoblastic diseases, and serum samples from 12 women with previous false-positive hCG results (primarily in the Abbott AxSYM assay) were blindly tested by commercial laboratories in the Beckman Access hCGβ, the Abbott AxSYM hCGβ, the Chiron ACS:180 hCGβ, the Baxter Stratus hCG test, the DPC Immulite hCG test, the Serono MAIAclone hCGβ tests, and in the hCGβ RIA.**

Results: **Only the RIA and the DPC appropriately detected the five irregular hCG standards. Only the Beckman, DPC, and Abbott assays gave results similar to the RIA in the patients with confirmed trophoblastic diseases (values within 25% of RIA in 49, 49, and 54 of 59 patients, respectively). For samples that were previously found to produce false-positive hCG results, no false-positive results were detected with the DPC and Chiron tests (5 samples, median <2 IU/L), but up to one-third of samples were false positive (>10 IU/L) in the Beckman (1 of 5), Serono (2 of 9), and Baxter assays (1 of 5), and the hCGβ RIA (3 of 9; median for all assays, <5 IU/L). These samples, which produced false-positive results earlier in the Abbott AxSYM assay, continued to produce high values upon reassessment (median, 81 IU/L).**

Conclusions: **Of six frequently used hCG immunometric assays, only the DPC detected the five irregular forms of βhCG, agreed with the RIA, and avoided false-positive results in the samples tested. This assay, and similarly designed assays not tested here, seem appropriate for hCG testing in the diagnosis and management of trophoblastic diseases.**

Human chorionic gonadotropin (hCG) is composed of two peptides, the α and β subunits, joined noncovalently. Approximately 30% of the molecular weight of hCG comprises carbohydrate side chains: four N-linked oligosaccharide and four O-linked oligosaccharides. The hCG α and β subunits are heterogeneous in both peptide and oligosaccharide side chain structures *(1, 2)*. Ordinary hCG (α-β dimer with no cleavages, monoantennary or biantennary N-linked oligosaccharides, and trisaccharide and tetrasaccharide O-linked oligosaccharides) is the principal form of hCG in serum during normal pregnancy. Ordinary hCG is accompanied by small and varying amounts of nicked hCG (cleaved on the β subunit, between residues 47 and 48), hyperglycosylated hCG (hCG with an abundance of larger or triantennary N-linked oligosaccharides and hexasaccharide O-linked oligosaccharides),

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nicked and hyperglycosylated hCG, free β subunit, nicked free β subunit, free α subunit, and large free α subunit (α subunit with triantennary N-linked oligosaccharides) *(1, 2)*. Commercial hCG (or hCGβ) assays all detect ordinary hCG, and to various extents detect nicked hCG, hyperglycosylated hCG, nicked and hyperglycosylated hCG, and nicked and nonnicked free β subunit. None of the commercial hCG (or hCGβ) methods detect either form of free α subunit *(2)*. With the preponderance of ordinary hCG in normal pregnancy serum, the ability of the assay to fully detect nicked hCG, hyperglycosylated hCG, nicked and hyperglycosylated hCG, and nicked and nonnicked free β subunit may make only a small difference to the immunoassay results and to the utility of the assay for pregnancy testing *(1, 3, 4)*.

Trophoblastic diseases include complete and partial hydatidiform mole, postmolar tumor, gestational choriocarcinoma, testicular choriocarcinoma, and placental site trophoblastic disease. These are also major sources of hCG. Precise hCG determinations are crucial in patients with trophoblastic diseases to assess the mass of tumor, the successful treatment of malignancy, or recurrence or persistence of disease *(3–5)*. In trophoblastic diseases, hyperglycosylated hCG, nicked hCG, hyperglycosylated and nicked hCG, or nicked or nonnicked free β subunit may be the principal source of immunoreactivity in serum *(2–7)*. Additional hCG variants are found in patients with trophoblastic diseases. These include nicked hCG missing the β subunit C-terminal peptide, residues 92–145, and alternatively nicked hCG (cleaved at β43–44 or β44–45) *(1)*. The inability of commercial hCG tests to fully detect these hCG variants has led to failure to detect persistent or recurrent trophoblastic diseases, requiring urgent chemotherapy or other surgery *(4, 8, 9)*.

Several centers have claimed that the competitive polyclonal anti-hCGβ RIA is the only type of assay that detects all of these variants equally and is the only test appropriate for monitoring trophoblastic diseases *(4, 7, 10, 11)*. For this reason, the hCGβ RIA has been proclaimed the "gold standard" for hCG testing in trophoblastic disease applications *(4, 7)*. A hCGβ RIA should not be mistaken for a hCG, hCGα, or hCGβ C-terminal peptide RIA, which may be much less broad in specificity. Other laboratories have found that newer immunometric assays and automated immunometric assays may also be appropriate for trophoblastic disease management or may even offer an improvement for this application *(6, 12–15)*. Our objective was to thoroughly examine the appropriateness of different hCG (or hCGβ) assays for monitoring patients with trophoblastic diseases. We examined the ability of six of the most commonly used automated immunometric assays to detect hCG and its variants in trophoblastic disease cases and to avoid unduly low or false-negative immunoassay values. Results were compared with a commercial laboratory's competitive hCGβ RIA, the gold standard.

A recent article identified 12 patients falsely treated for presumed choriocarcinoma because of false-positive hCG results *(16, 17)*. Other reports have also identified patients erroneously diagnosed with choriocarcinoma because of false-positive hCG results *(9, 18, 19)*. False-positive hCG results have also been identified in three patients after evacuation of complete hydatidiform mole (Cole LA and Butler SA, unpublished data). This can lead to false diagnosis of a postmolar tumor. A recent report from the Trophoblastic Disease Center in London noted that a significant proportion of choriocarcinoma cases are presumed solely on the basis of persistently increased hCG results *(20)*. Most cases of postmolar tumor are assumed on the basis of increased hCG results after evacuation of a complete hydatidiform mole. Our objective was to also examine the accuracy of hCG (or hCGβ) assays for diagnosing patients with choriocarcinoma or postmolar tumor. Samples that had been found to produce false-positive results previously *(16, 17, 19)* were examined using the same six automated immunometric hCG (or hCGβ) tests and the hCGβ RIA.

Materials and Methods

Serum samples were collected at Charing Cross Hospital, London, from 50 women, and from other centers throughout the world from an additional 9 women with confirmed trophoblastic diseases. Of the 59 women, 8 had pathology-confirmed choriocarcinoma, and 51 had complete hydatidiform mole. Of the latter, 30 provided serum samples for this study preevacuation of the mole or in the 2 weeks following evacuation (hCG >500 IU/L), and 21 provided a sample in the later weeks following evacuation of the mole (2–6 weeks; hCG <500 IU/L) when the hCG concentration was slowly decreasing.

In addition to these 59 serum samples, five standards were prepared from pure hCG preparations. These were pure hyperglycosylated hCG, pure nicked hCG, pure asialo hCG (hCG missing sialic acid residues on N- and O-linked oligosaccharides), pure hCG minus the C-terminal peptide, and nonnicked free β subunit. These were purified and prepared from pregnancy, complete hydatidiform mole, or choriocarcinoma urine samples, as described previously *(1)*. Peptide and oligosaccharide structures had been determined to fully characterize the preparations *(1)*.

Additional serum samples were collected from 12 women erroneously diagnosed as having choriocarcinoma or gestational trophoblastic disease because of false-positive hCG results *(16, 17)*. In all 12 cases, the false-positive results were seemingly attributable to the presence of heterophilic antibodies in blood (Cole LA and Shahabi S, unpublished data). All 12 women were shown to lack measurable concentrations (>2 IU/L) of true hCG or its breakdown products *(16, 17)*. The 12 serum samples were those sequentially accrued by the hCG Reference Service and were not selected in any way. Ten of the 12 patients were falsely monitored by their physicians, using the Abbott AxSYM hCGβ test, 1 patient was monitored

using both the Abbott AxSYM and IMx hCGβ tests, and 1 was monitored using the Bayer Immuno-1 hCGβ assay.

On the basis of the hCG results supplied by Charing Cross Hospital and patient treatment centers, the 59 confirmed trophoblastic disease serum samples were each diluted with normal male serum (Sigma) to ~200 IU/L (samples with hCG <200 IU/L were not diluted). The five hCG structure standards were calibrated by amino acid analysis. Results were converted to IU/L hCG, or molar equivalents of hCG based on the molecular weights of hCG and its β subunit *(21)*, using the formula established by the World Health Organization for the preparation of the First International Reference Preparation and the Third International Standard for immunoassays (1 μg of pure hCG = 9.3 IU) *(22)*. Standards were diluted in normal male serum to a concentration of 200 IU/L or the molar equivalent (0.57 pmol/L hCG).

The trophoblastic disease patient samples, the 5 standards, and 4 of the 12 false-positive serum samples (only 4 samples were available at the time of initial coding), a total of 68 samples, were mixed, coded, and sent to independent commercial laboratories for blind testing. The remaining eight false-positive samples were tested by commercial laboratories at later times, when samples became available.

Samples were tested by four commercial laboratories. The Chemistry Laboratory in the Department of Pathology at Hurley Medical Center (Flint, MI) blindly tested coded samples in the Beckman Access hCGβ (total hCG), the Abbott AxSYM hCGβ (total hCG), the Chiron ACS:180 hCGβ (total hCG), and the Baxter Stratus hCG test (intact hCG). This was carried out by Dr. Harland Verrill as part of a collaboration, without charge. Two of the samples were tested by the Abbott AxSYM hCGβ assay at Quest Diagnostics (Teterboro, NJ). The Endocrinology Laboratory at Yale-New Haven Hospital (New Haven, CT) blindly tested the coded samples in the Serono MAIAclone hCGβ (total hCG) and in their tumor marker hCG test, a goat polyclonal anti-hCGβ competitive RIA (total hCG test; 3-day assay using rabbit polyclonal anti-β antisera and ^{125}I-hCG tracer, bound material precipitated with goat anti-rabbit γ-globulin). A standard immunoassay fee was paid for this service. Finally, the DPC Immulite hCG test (total hCG) was run blindly at the laboratories of Diagnostic Products Corp. (Los Angeles, CA) without charge. This is an independent research study. The authors received no funds from, and have neither personal interest in nor conscious bias for or against any of the described manufacturers.

Results were decoded and entered into a Microsoft Excel spreadsheet. The six automated immunometric assay hCG/hCGβ assay values were compared with the hCGβ RIA results, and we tabulated the number of samples in which results were >25% below or >25% above the RIA result. Because individual hCG values usually are logarithmically distributed *(23, 24)*, the median results are presented.

Results

Seventy-six serum samples (5 hCG variant standards, 8 samples from patients with choriocarcinoma, 51 from those with complete hydatidiform mole, and 12 from those with confirmed false-positive hCG results) were tested by independent laboratories in six automated immunometric hCG/hCGβ assays and by an hCGβ RIA.

The six examined assays varied in their recognition of purified (200 IU/L) hCG variants (Table 1). The Serono MAIAclone hCGβ assay ineffectively recognized free β subunit (3.8% recovery), hyperglycosylated hCG (3.9%), and nicked hCG (27%). The Baxter Stratus hCG test did not detect free β-subunit standard (<1% recovery). The Chiron ACS:180 hCGβ test underdetected free β-subunit standard (70%). Three tests, the Beckman Access, Abbott AxSYM, and Chiron ACS:180, did not detect the hCG minus C-terminal peptide (<1% recovery). The commercial hCGβ RIA (the gold standard) and the DPC Immulite hCG automated immunometric assay were the only tests with apparent recoveries of at least 75% for all five hCG-related molecule standards.

We examined the detection of hCG and related molecules in serum samples from patients with complete hydatidiform mole. Samples from 30 patients were collected before and up to 2 week after evacuation of a complete hydatidiform mole (Table 2). Additional sam-

Table 1. Ability of hCG immunoassays to measure hCG metabolic products[a] commonly found in individuals with trophoblastic disease or choriocarcinoma.

Standard[d] (molar equivalent of 200 IU/L)	Commercial hCGβ RIA, IU/L	Beckman Access hCGβ, IU/L	Abbott AxSYM hCGβ, IU/L	Chiron ACS:180 hCGβ, IU/L	DPC Immulite hCG, IU/L	Baxter Stratus hCG, IU/L	Serono MAIAclone, IU/L
Free β-subunit	171	356	200	**140**	234	**<2**	**7.6**
Hyperglycosylated hCG	184	255	181	234	207	189	**7.8**
Nicked hCG (cleaved β47–48)	195	224	242	194	235	169	**54**
Asialo hCG	248	175	243	162	285	191	185
hCG minus C-terminal peptide	190	**<2**	**<2**	**<2**	273	190	**147**
Number of low results (<150 IU/L)	0 of 5	1 of 5	1 of 5	2 of 5	0 of 5	1 of 5	4 of 5

[a] The precise structures of these preparations have been published *(1)*. All standards were calibrated by amino acid analysis and converted into the international units, based on molecular weight and molar equivalents of hCG. Samples were coded and tested blindly by multiple external laboratories as described in *Materials and Methods*. Unduly low (<150 IU/L of 25% below calibrated concentration) results are in bold.

Table 2. Analysis of serum from 30 patients before and up to 2 weeks after evacuation of a complete hydatidiform mole (hCG >500 IU/L total).[a]

Total hCG concentration,[b] IU/L	Dilution supplied to test centers	Commercial hCGβ RIA, IU/L	Beckman Access hCGβ, IU/L	Abbott AxSYM hCGβ, IU/L	Chiron ACS:180 hCGβ, IU/L	DPC Immulite hCG, IU/L	Baxter Stratus hCG, IU/L	Serono MAIAclone, IU/L
560	5	112	123	121	121	112	91	112
740	10	74	84	81	86	73	69	80
760	10	76	97	111	90	83	66	75
780	10	78	92	72	100	77	76	86
800	10	80	93	70	94	75	67	80
1020	5	204	220	197	223	206	179	220
1210	10	121	137	134	147	110	108	121
1420	10	142	162	157	150	130	**100**	128
1800	10	180	209	184	218	174	159	187
2700	30	90	112	101	115	86	76	85
3630	30	121	128	124	137	108	105	122
3930	30	131	142	146	154	125	102	122
4250	10	425	**284**	353	**245**	**219**	**130**	**171**
4560	60	76	99	83	100	83	82	91
5340	30	178	176	164	191	149	150	175
5640	60	94	110	98	115	90	93	105
6120	60	102	107	114	111	96	90	103
6810	30	227	245	236	245	214	181	222
6900	60	115	137	119	152	108	105	123
8100	30	270	279	245	283	241	237	264
8250	150	55	51	45	59	59	48	53
10 560	60	176	203	165	214	167	164	179
12 750	150	85	106	88	**142**	94	88	99
14 700	150	98	132	110	**162**	110	105	122
16 200	150	108	139	114	**178**	119	110	121
23 850	150	159	174	154	213	140	133	144
25 000	1000	25	**41**	36	**50**	35	33	35
27 150	150	181	262	239	**284**	220	201	225
44 000	400	110	129	115	162	107	103	121
54 400	400	136	174	163	209	143	146	168
Median result		114	134	120	151	110	104	122
Total number varying >25% from RIA result			2 of 30	0 of 30	6 of 30	1 of 30	2 of 30	1 of 30

[a] Samples were tested for hCG in a RIA, and results were compared with those for 6 immunometric assays. Samples were diluted to 200 IU/L based on the Charing Cross house immunoassay, coded, and tested blindly by multiple external laboratories as described in *Materials and Methods*. Results varying >25% from commercial hCGβ RIA values are indicated in bold.

[b] As determined from dilution and commercial hCGβ RIA.

ples were collected from 21 patients at 2–6 weeks after evacuation of hydatidiform mole (Table 3), and from 8 women with choriocarcinoma (Table 4). Results from the six automated immunometric assays were compared with those from the commercial laboratory's hCGβ RIA. For the purposes of this study, errors were considered as results deviating from the RIA value by >25%. The fewest errors were found with the Abbott AxSYM hCGβ test (5 errors in 59 patients total; 8.5%) and with the DPC Immulite hCG and Beckman Access hCG (10 errors total; 17%). More errors were found with the Serono MAIAclone (14 errors total; 24%) and with the Chiron ACS:180 hCGβ (16 errors total; 27%). The most errors were noted with the Baxter Stratus hCG (22 errors total; 37%).

Most of the errors were noted in samples taken 2–6 weeks after evacuation of hydatidiform mole (Table 3) and with choriocarcinoma serum samples (Table 4). These errors were particularly evident with the Chiron ACS:180 hCGβ test (errors in 5 of 21 mole and 5 of 8 choriocarcinoma cases), the Serono MAIAclone assay (errors in 7 of 21 mole and 6 of 8 choriocarcinoma cases), and the Baxter Stratus hCG assay (errors in 15 of 21 mole and 5 of 8 choriocarcinoma cases).

We also examined serum from 12 patients with a history of false-positive hCG results (Table 5). These were tested in the commercial RIA and in the six automated immunometric assays. Insufficient serum was available to test all samples in each of the seven assays. We considered false-positive results >10 IU/L for diagnosis of trophoblastic disease (or pregnancy). Concentrations up to 10 IU/L hCG can be produced by the pituitary and other

Table 3. Analysis of serum from 21 patients with low hCG 2–6 weeks (<500 IU/L total) after evacuation of complete hydatidiform mole.[a]

Total hCG concentration,[b] IU/L	Dilution supplied to test centers	Commercial hCGβ RIA, IU/L	Beckman Access hCGβ, IU/L	Abbott AxSYM hCGβ, IU/L	Chiron ACS:180 hCGβ, IU/L	DPC Immulite hCG, IU/L	Baxter Stratus hCG, IU/L	Serono MAIAclone, IU/L
5.0	1	5.0	**8.7**	7.1	4.6	5.9	**3.2**	5.3
10	1	10	11	10	**7.1**	9.2	8.8	12
12	1	4	5.8	5.1	3.3	4	**<2**	**12**
12	1	12	15	16	14	13	12	16
13	1	13	18	18	14	11	**5.7**	**8.5**
14	1	14	**10**	**10**	**6.6**	**8.8**	**6.9**	**8.1**
17	1	17	16	16	**11**	**11**	**7.1**	14
26	1	26	25	26	22	**19**	**15**	24
30	1	30	36	40	30	29	**15**	24
59	1	59	65	70	67	**115**	91	79
60	2	30	36	43	32	24	**17**	**22**
62	2	31	45	46	44	35	28	33
94	2	47	61	**76**	52	55	**34**	41
124	2	62	**45**	**44**	**41**	**41**	**30**	**31**
132	2	66	88	99	80	73	53	66
162	1	162	**31**	**48**	**15**	235	**<2**	**12**
175	5	35	47	46	51	41	43	53
216	2	108	107	123	94	103	**69**	86
236	2	118	128	146	130	112	**86**	106
246	2	123	121	111	113	**92**	**32**	**50**
400	5	80	85	93	70	75	**60**	75
Median result		31	36	44	32	35	28	24
Total number varying >25% from RIA result			4 of 21	4 of 21	5 of 21	6 of 21	15 of 21	7 of 21

[a] Samples were tested for hCG in a RIA, and results were compared with those for 6 immunometric assays. Samples were diluted to 200 IU/L based on the Charing Cross house immunoassay, coded, and tested blindly by multiple external laboratories as described in *Materials and Methods*. Assay results that were reported as 0, <1, or <2 IU/L are marked as <2 IU/L. Results varying <25% from commercial hCGβ RIA values are indicated in bold.

[b] As determined from dilution and commercial hCGβ RIA.

sources *(3, 10, 12)*. None of the samples tested exceeded 10 IU/L in the DPC Immulite hCG or the Chiron ACS:180 hCGβ test (0 of 6 and 0 of 5 samples, respectively). Less than one-third of the samples tested exceeded 10 IU/L in the Baxter Status hCG, Beckman Access hCGβ, and Serono MAIAclone hCGβ assays, or in the commercial hCGβ RIA (1 of 5, 1 of 5, 2 of 9, and 3 of 9 samples, respectively). Because most of these samples were known to produce false-positive results in the Abbott AxSYM assay, we expected and found false-positive results in the Abbott AxSYM hCGβ test (10 of 10 samples).

To evaluate the extent of false positivity, we calculated

Table 4. Analysis of serum samples from 8 patients with choriocarcinoma by an hCGβ RIA and comparison with results from 6 hCG immunometric assays.[a]

Total hCG concentration,[b] IU/L	Dilution supplied to test centers	Commercial hCGβ RIA, IU/L	Beckman Access hCGβ, IU/L	Abbott AxSYM hCGβ, IU/L	Chiron ACS:180 hCGβ, IU/L	DPC Immulite hCG, IU/L	Baxter Stratus hCG, IU/L	Serono MAIAclone, IU/L
75	1	75	107	98	109	85	82	**235**
99	1	99	128	126	102	101	78	96
120	1	120	**244**	115	**87**	**182**	**19**	**9**
580	10	58	84	60	**96**	55	69	61
11 940	30	398	**213**	319	**248**	**544**	**267**	**253**
131 000	1000	131	170	121	130	126	**67**	**75**
229 000	1000	229	**122**	185	**68**	200	**81**	**89**
3 456 000	16000	216	**11**	**14**	**6.9**	**55**	**109**	**19**
Median result		126	125	118	99	114	79	82
Total number varying >25% from RIA result			4 of 8	1 of 8	5 of 8	3 of 8	5 of 8	6 of 8

[a] Samples were diluted to 200 IU/L based on the Charing Cross house immunoassay, coded, and tested blindly by multiple external laboratories as described in *Materials and Methods*. Results varying >25% from commercial hCGβ RIA values are indicated in bold.

[b] As determined from dilution and commercial hCGβ RIA.

Table 5. Analysis of serum samples from 12 patients erroneously diagnosed with choriocarcinoma or gestational trophoblastic disease based on false-positive hCG data (*16*).[a]

	Commercial hCGβ RIA, IU/L	Beckman Access hCGβ, IU/L	Abbott AxSYM hCGβ, IU/L	Chiron ACS:180 hCGβ, IU/L	DPC Immulite hCG, IU/L	Baxter Stratus hCG, IU/L	Serono MAIAclone, IU/L
	ND[b]	ND	ND	ND	ND	**53**	ND
	ND	ND	**150**	ND	ND	ND	ND
	ND	ND	ND	ND	<2	ND	ND
	<2	ND	**17**	ND	ND	ND	<2
	28	**30**	**14**	9.7	ND	<2	<2
	<2	<2	**32**	<2	<2	<2	**133**
	<2	ND	**33**	ND	<2	ND	<2
	<2	4.6	**68**	<2	<2	<2	2.0
	<2	ND	**93**	ND	ND	ND	3.5
	10	6.6	**110**	4.5	4.19	2.6	**13**
	11	ND	**145**	ND	ND	ND	3.5
	11	<2	**175**	<2	<2	ND	7.8
Median reported concentration	<2	4.6	81	<2	<2	<2	3.5
>10 IU/L reported	3 of 9	1 of 5	10 of 10	0 of 5	0 of 6	1 of 5	2 of 9

[a] Samples were tested for hCG by the hCGβ RIA and 6 immunometric assays. Samples were coded and tested blindly by multiple external laboratories as described in *Materials and Methods.* Assay results reported by testing centers as 0, <1, or <2 IU/L were considered at the limit of detection and were recorded as <2 IU/L. False-positive values exceeding 10 IU/L are indicated in bold. All samples were supplied to test centers undiluted.

[b] ND, not determined. Insufficient serum available to run test.

the median concentrations. The median result was <2 IU/L in four of the assays and <5 IU/L in two other assays. In the Abbott AxSYM hCGβ test, however, the median was 81 IU/L.

Discussion

All quantitative serum hCG assays sold in the United States have a warning in their package insert noting that they are approved by the Food and Drug Administration (FDA) only for, and have been tested only for, normal pregnancy testing applications. Today, however, a high proportion of hCG tests run by all clinical laboratories are for non-FDA-approved or other applications. These include second-trimester Down syndrome screening, detection of ectopic pregnancies, management of hyperemesis gravidarum, diagnosis and management of trophoblastic diseases, and diagnosis and management of testicular and germ cell neoplasms. More than 50 different quantitative serum hCG tests are used today in clinical laboratories throughout the United States. All are used, without hesitation or question about hCG specificity, for anything from pregnancy detection to monitoring chemotherapy for choriocarcinoma *(2, 4, 5, 10, 25)*.

Textbooks on obstetrics and gynecology emphasize the essentiality of hCG testing in patients with trophoblastic diseases. This is a mandated application, although it is not approved by the FDA and has not been formally tested by hCG assay manufacturers. hCG is the vital test in the identification of choriocarcinoma, differentiating this cancer from others. It acts as a perfect tumor marker (100% sensitivity) for managing the treatment for choriocarcinoma and for detecting recurrences of disease *(18, 25)*. Although hCG testing is not essential in the diagnosis of hydatidiform mole, it is essential for demonstrating the complete removal of molar tissue and for the rapid identification of postmolar tumor or persistent trophoblastic disease *(26)*. Problems have been reported in the use of hCG immunoassays for both detecting (false-positive results) and monitoring the progress of (false-negative or unduly low results) trophoblastic diseases *(2–8, 10)*. In this study, we examined the performance of the competitive hCGβ RIA (the gold standard hCG test for trophoblastic diseases) and six of the most commonly used automated immunometric assays in diagnosing and monitoring hydatidiform mole and choriocarcinoma.

Hyperglycosylated hCG, nicked hCG, hCG minus C-terminal peptide, asialo hCG, and free β subunit are either unique to trophoblastic diseases or more abundant in trophoblastic disease samples *(1, 2–7)*. Only the hCGβ RIA and the DPC test effectively detected all of the hCG breakdown products or glycosylation variants. Not surprisingly, three tests, the Beckman, Abbott, and Chiron assays, did not detect hCG minus C-terminal peptide. The C-terminal peptide is a commonly used hCG epitope because it is unique to the β subunit of hCG and is not present on luteinizing hormone. Other β-subunit core epitopes can be targeted to effectively differentiate hCG and luteinizing hormone *(2)*.

Considering the assay specificities, we examined serum from 59 patients with confirmed trophoblastic diseases. Results were compared with the hCGβ RIA. Three tests (the Abbott, the DPC, and the Beckman) performed well, with few important differences in results compared with the hCGβ RIA. For three of the six tests (the Serono, Chiron, and Baxter tests), however, 24%, 27%, and 37% of results compared poorly with the hCGβ RIA. Errors were

most evident with these three assays in serum samples from women 2–6 weeks after evacuation of hydatidiform mole and in samples from patients being monitored for choriocarcinoma (34%, 45%, and 69% errors, respectively). These are clinical situations in which accurate hCG measurements are needed. We infer that theses three assays, and other similar assays not tested in this study, should probably be avoided in the management of trophoblastic disease. These three assays (Serono, Chiron, and Baxter) have minimal or less than normal detection of free β subunit. This is the likely cause of the poor performance in samples from patients with trophoblastic diseases. Clearly, equimolar detection of hCG and free β subunit is important in the management of trophoblastic diseases *(2–7)*. The Serono test also poorly detected nicked hCG and hyperglycosylated hCG, which are also important in the management of trophoblastic diseases *(1, 2, 4)*.

In four individual cases (patients with total hCG concentrations of 14, 124, 162, and 3 456 000 IU/L; see Tables 3 and 4), unduly low results were found with three other assays (Beckman, Abbott, and Chiron). These three methods did not detect hCG minus C-terminal peptide. Detection of this hCG degradation product may also be important in the management of patients with trophoblastic diseases.

Patients can be diagnosed with choriocarcinoma solely from a persistent positive hCG result, in the absence of an imaged tumor or a pregnancy *(16, 18)*. In most cases, chemotherapy or surgery is initiated solely because of persistent positive hCG results *(16)*. False-positive hCG results can occur in the hCG assay, and patients have been erroneously diagnosed and treated for choriocarcinoma. The hCG Reference Service has now identified 20 such false choriocarcinoma cases; 14 needlessly received surgery or chemotherapy [Ref. *(16)* and Cole LA, unpublished data]. False-positive results have also been observed in three patients after evacuation of hydatidiform mole (Cole LA and Butler SA, unpublished data). False-positive hCG results typically come from heterophilic antibodies present in human serum *(9, 16, 18, 27)*. We used such sera from the initial 12 patients identified with false-positive hCG results at the hCG Reference Service *(16, 17)* to further evaluate the hCGβ RIA and the six automated immunometric tests.

The DPC and Chiron assays produced no false-positive results (<10 IU/L). Up to one-third of samples gave false-positive results with the hCGβ RIA, and the Beckman, Baxter, and Serono assays. It is important to note that 11 of the 12 sera were from patients who had been tested earlier using the Abbott AxSYM test. In fact, 19 of the 20 patients identified to date by the hCG Reference Service with false-positive hCG had been monitored by their physicians using the Abbott AxSYM test [Ref. *(16)* and Cole LA, unpublished data]. It is no surprise that 10 of 10 samples evaluated here were falsely positive in this assay. The median false-positive hCG result was 81 IU/L in the Abbott test, and <5 IU/L in all other tests. Such (lower) false-positive results found in the other assays are less likely to meet the threshold for the assumed diagnosis of choriocarcinoma and for therapy *(16)*. The finding of multiple serum samples positive in the Abbott test and serum samples producing lower or negative results in other assays suggests a potential problem with this test.

We have examined the specificity of hCG immunoassays for detecting variant forms of hCG present in trophoblastic diseases, compared the utility of automated hCG immunometric assays with the hCGβ RIA in the management of trophoblastic diseases, and have examined the potential of hCG assays to give false-positive results. Only the DPC gave consistently acceptable results in all three categories. The DPC test is a chemiluminescence test, using a capture antibody and a tracer antibody directed toward different regions of the core of hCG β subunit. Several other commercial tests use similar antibody arrangements *(2)*. These assays might be similarly utilized in the management of trophoblastic diseases. The DPC and similarly designed tests may be as useful as hCGβ RIA in the management of trophoblastic diseases. One must consider the negative aspects of an hCGβ RIA, including its complexity, the extensive pipetting, its relative imprecision, and its time-consuming procedures.

The DPC test and the hCGβ RIA both appropriately recognized all of the common hCG metabolic products associated with trophoblastic diseases, but the DPC test yielded results that differed from the hCGβ RIA data in 10 of 59 patients with trophoblast disease. In 7 of these 10 cases, the DPC result was close to the mean result for the 7 assays evaluated (within 25%; data not shown), but not close to the hCG RIA data. Thus, the error may lie with the RIA result rather than with the DPC values. The DPC and other similarly designed tests might today be the tests of choice for diagnosing and monitoring trophoblastic diseases and for other applications requiring careful detection of true-positive hCG and recognition of both intact hCG and hCG metabolic products.

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